Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 7079–7083, October 1988
Biochemistry

BK

Oligodeoxynucleoside phosphoramidates and phosphorothioates as inhibitors of human immunodeficiency virus

(antisense oligonucleotides/antiviral drugs/competitive hybridization/hybridon)

SUDHIR AGRAWAL*, JOHN GOODCHILD*, MARIA P. CIVEIRA†, ARTHUR H. THORNTON†, PREM S. SARIN†, AND PAUL C. ZAMECNIK*

*Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545; and †Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20892

Contributed by Paul C. Zamecnik, June 3, 1988

ABSTRACT **Modified oligodeoxynucleotides complementary to RNA of human immunodeficiency virus 1 (HIV-1) were tested for their ability to inhibit virally induced syncytium formation and expression of viral p24 protein. The modifications of oligomers include replacement of backbone phosphodiester groups with phosphorothioates and various phosphoramidates. All oligomers were found to be active. Oligomers with complete replacement of phosphodiesters with phosphoramidate or phosphorothioate groups were more active at the micromolar range than were unmodified oligomers of the same sequence. In addition, modified and unmodified homooligonucleotides also showed inhibition of HIV-1 replication. It is suggested that different classes of oligonucleotides may inhibit HIV replication by different mechanisms.**

Oligonucleotides complementary to viral RNA have been shown to inhibit viral replication in cell cultures with Rous sarcoma virus (1), human immunodeficiency virus (HIV) (2–4), vesicular stomatitis virus (5–7), herpes simplex virus 1 (5, 8), and influenza virus (9). Similarly, the expression of the c-*myc* protooncogene has been inhibited in HL-60 cells and T lymphocytes (10–12, 22) by oligonucleotides complementary to mRNA. Oligonucleotides are taken up by cells by a process that appears, at least in some cases, to require energy (4). This "antisense" approach to the control of RNA expression has potential for therapeutic use in the treatment of patients with acquired immunodeficiency syndrome (AIDS). Limiting factors for the therapeutic use of oligonucleotides could include degradation of the oligonucleotides by nucleases and low cellular uptake of polyanions of this type. To overcome these problems, some of the antisense oligonucleotides have been modified on the backbone as methylphosphonates (5, 8) or phosphorothioates (3). This field has recently been reviewed by Stein and Cohen (9).

We demonstrated that unmodified oligonucleotides could be used to inhibit HIV (2, 4). In unpublished results we have found inhibition of HIV by oligonucleoside methylphosphonates. Here, we report the synthesis of oligonucleoside phosphoramidates and phosphorothioates and their anti-HIV activity. The structures of these modified internucleoside linkages are given in Fig. 1. All the modified oligonucleotides were found to be resistant to nucleases such as snake venom or spleen phosphodiesterases. Oligonucleoside phosphorothioates have been shown by Matsukura *et al.* (3) to inhibit HIV replication.

MATERIALS AND METHODS

Oligonucleotides were synthesized on an automated synthesizer (model 8600, Biosearch, San Rafael, CA). Larger amounts of material for toxicity studies were made by a manual solid-support method on an Omnifit assembly (13). Unmodified oligomers were made and purified by methods described previously (2). Phosphorothioates and phosphoramidates were synthesized by a modification of the H-phosphonate procedure (14, 15). To generate phosphorothioate linkages, oxidation following the final coupling and detritylation was replaced by treatment with 0.1 M sulfur in carbon disulfide/triethylamine (9:1, vol/vol) at room temperature for up to 2 hr, depending on chain length (16). For the phosphoramidates, this step was replaced by treatment with a 10% solution of the appropriate amine in carbon tetrachloride for up to 1.5 hr, depending on chain length (16).

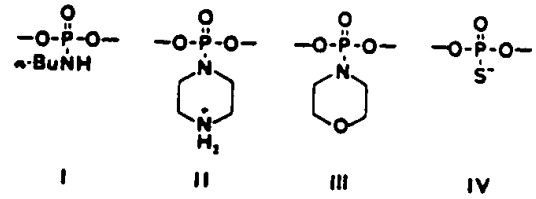

FIG. 1. Internucleoside linkages used in the study. The phosphoramidates are butylamidate (I), piperazidate (II), and morpholidate (III). The phosphorothioate is structure IV.

After deblocking in concentrated ammonium hydroxide (55°C for 5½ hr or room temperature for >2 days), products were purified on 2-mm-thick preparative layer plates (Merck silica gel 60) in propanol/water/concentrated ammonium hydroxide (55:35:10, vol/vol). Phosphorothioates were further purified on DEAE-cellulose and C_{18} silica (details will be published elsewhere). Phosphoramidates from preparative layer plates were passed through Sephadex G-25 in aqueous 30% (vol/vol) ethanol and then through C_{18} silica and dialyzed.

In all cases, small amounts of oligomers attached to the solid support were taken before the final treatment with sulfur or amine and were oxidized with 2% iodine in pyridine/water (98:2, vol/vol) (15) to phosphodiesters. These were used for determination of base composition by enzymatic degradation to nucleosides followed by HPLC. The phosphorothioates were also characterized by polyacrylamide gel electrophoresis, where they had similar mobility to the corresponding diesters.

Phosphoramidates were further characterized by hydrolysis with formic acid to the phosphodiesters (16) for comparison with authentic samples on HPLC and degradation to nucleosides for determination of nucleoside composition and integrity by HPLC.

A useful indicator of oligonucleotide purity and integrity is the melting curve of its duplex with a complementary DNA

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome.

strand. The presence of shorter impurities produced broadening of the curve, whereas backbone modifications reduced the melting temperature. The amidates generally had lower melting temperatures than the phosphorothioates; an exception appears to be *N*-methylphosphoramidate.

Assays for HIV-1 Inhibition. The inhibition of HIV-1 expression in H9 or MOLT-3 cells in the presence of antisense oligonucleotides was carried out by infecting 5×10^5 cells per ml with $2.5–5 \times 10^4$ virus particles of HIV-1 strains HTLV-IIIB or HTLV-IIIC. Infection with 500–1000 virus particles per cell represents a multiplicity of infection (MOI) of 0.5–1. HIV-1 infection of cells was carried out by simultaneous addition of virus and antisense oligomers to the cells in culture. The cultures were incubated in culture medium containing RPMI 1640, 10% (vol/vol) fetal bovine serum, 2 mM glutamine, and 250 μg of gentamicin per ml, in a humidifed atmosphere containing 5% CO_2 at 37°C. After 4 days, the cells and supernatant were examined for the level of HIV-1 expression by measuring syncytia (MOLT-3 cells) and viral antigen expression as well as cell viability. The number of syncytia formed in MOLT-3 cells were counted after triturating the cells to obtain an even distribution of the syncytia in the culture. The average number of syncytia was obtained by counting several fields in duplicate cultures. Cell viability was measured in the presence of trypan blue, and the cells that excluded the dye were counted as viable cells. HIV-1 antigen expression was measured in cells fixed in methanol/acetone as described (17, 18). In brief, the cells were pelleted and then resuspended in phosphate-buffered saline (PBS) at a concentration of 10^6 cells per ml. The cells were spotted on toxoplasmosis slides, air-dried, and fixed in methanol/acetone (1:1, vol/vol) for 15 min at room temperature. The slides were next incubated with 10% normal goat serum at room temperature for 30 min and washed with PBS (four times). HIV-1 p24 or p17 monoclonal antibody was added to each well and the slides were incubated for 30 min in a humid chamber at 37°C. The slides were washed with PBS (four times), incubated with fluorescein isothiocyanate-labeled goat anti-mouse IgG (Cappel Laboratories, Cochranville, PA) for 30 min at 37°C, and then washed with PBS overnight. The slides were counterstained with Evan's blue, washed with PBS, mounted with 50% glycerol, and examined with a Zeiss fluorescence microscope. The percentages of cells fluorescing in the oligomer-treated and untreated cultures were compared. Inhibition of HIV-1 expression in the presence of oligomers was found to be similar in both the H9 and the MOLT-3 cells.

RESULTS

Anti-HIV Activity of Oligonucleoside Phosphorothioates. Oligonucleotides with chain lengths of 15 or 20 bases, complementary to three sites in HIV RNA previously shown to be good targets for inhibition (ref. 4 and unpublished results), were tested for inhibition of HIV replication. These sites were the capped end of viral RNA, a splice donor site near the 5′ end of the RNA used to assemble shorter mRNAs, and a splice acceptor site used to generate *tat* mRNA. All the antisense oligomers inhibited HIV in cell culture (Table 1). Two oligonucleoside phosphorothioates of chain length 15 (compounds 3 and 6 in Table 1) were less active than those with chain length 20 (compounds 4 and 5). In particular, compounds 5 and 6 should be compared, as they share a common sequence. The phosphorothioates typically are more active than the diester series, but *for an accurate comparison, all compounds should be tested simultaneously.* An unmodified oligomer with the same sequence as compound 5 and tested at the same time at a concentration of 50 μg/ml showed 77% inhibition of syncytium formation and 72% inhibition of p24 expression. These values are lower than the activity of the phosphorothioate analogue at 20 μg/ml (3 μM).

In another experiment, compound 5 was given 4 days after addition of the virus and incubation was continued for a further 4 days before expression of p24 was assayed. Although inhibition was somewhat lower than when the compound was given simultaneously with the virus, it was still high. At drug concentrations of 12.5, 25, 50, 100, and 200 μg/ml, the percentages of inhibition were, respectively, 4%, 19%, 70%, 82%, and 86%.

Included in this series were two phosphorothioate derivatives not complementary to HIV RNA. One of these (compound 2) showed no activity up to 100 μg/ml. This compound, however, is self-complementary, and complementary oligodeoxynucleoside phosphorothioates have been shown to hybridize (20). Another compound (no. 1), complementary to neither itself nor HIV RNA, showed no activity at 4 μg/ml but at 20 μg/ml was equally active as the antisense phosphorothioates, but also showed some toxicity. This suggests that at higher concentrations, these compounds may work by a mechanism other than antisense inhibition. A similar conclusion was reached by Matsukura *et al.* (3).

Anti-HIV-1 Activity of Oligonucleoside Phosphoramidates. Three classes of phosphoramidates were studied (Fig. 1) and the same target sites on HIV RNA were used as with the phosphorothioates. As with the phosphorothioates and diesters, oligomers with chain length of 20 were more active than those of 15 sharing a common sequence (Table 1; cf. compounds 8 and 9, 10 and 11, and 12 and 13). In one pair the longer oligomer was also more toxic than the shorter oligomer. Apparently, activity is not critically dependent on the nature of the phosphoramidate group (cf. compound 8 with 12 and 14 and compound 10 with 15).

Anti-HIV Activity of Homooligodeoxynucleotides. In agreement with an earlier report (3), we found some homooligonucleoside phosphorothioates to be at least as active as the antisense sequences. We have extended the study of homooligonucleotides to include phosphodiesters and phosphormorpholidates. All these compounds were active, and activity was of the same order as for antisense heterooligomers (Table 2). In the three classes of backbones used, the dT and dG homosequences were consistently more active than dC or dA when ranked by summing total activities. This observation is somewhat different from the previous report of phosphorothioates alone, where dC_{14} was found to be most active (3). In general, the toxicity of homooligomers was greater than that of antisense oligomers as measured by reduction in cell number (Tables 1 and 2; unpublished results).

Toxicity of Oligodeoxynucleoside Phosphorothioates in Mice. Preliminary acute toxicity studies in mice were performed with compound 5 at a concentration of 640 μg/ml in pyrogen-free distilled water. Two male and two female mice were injected intraperitoneally for each dose of compound. No symptoms of toxicity were observed for up to 14 days after injection of 2.5, 10, or 40 mg/kg of body weight. With a dose of 160 mg/kg, one animal died on day 3; with a dose of 640 mg/kg, all four animals died within 1 hr. These results show a relatively low degree of toxicity and are very similar to those obtained in acute toxicity studies with the parent phosphodiester compound (23).

DISCUSSION

The phosphate modifications used in this study gave oligonucleotides with backbones that were negatively charged (phosphorothioate), uncharged (phosphormorpholidate, *N*-butylphosphoramidate), or positively charged (phosphorpiperazidate). These compounds were more active than the parent diesters (Fig. 2). The nature of the backbone was not

Table 1. Inhibition of HIV replication by backbone-modified oligodeoxynucleotides

Compound no.	Sequence	Binding site*	Conc., μg/ml	% inhibition†		% reduction in cell no.‡
				Syncytia	p24	
		Phosphorothioates				
1	CGAGATAATGTTCACACAAC	None§	4	0	0	2
			20	89	93	35
			100	100	100	67
2	ACGTACGTACGTACGTACGT	None§	5	0	0	
			29	0	0	
			50	0	0	
			100	0	0	
3	CTAACCAGAGAGACC	1–15	4	13	5	0
			20	29	28	32
			100	58	24	43
4	GCGTACTCACCAGTCGCCGC	280–299	4	77	56	
			20	99	98	
			100	100	100	
5	ACACCCAATTCTGAAAATGG	5349–5368	4	38	63	0
			20	86	91	0
			100	100	100	0
6	CCCAATTCTGAAAAT	5351–5365	4	14	39	0
			20	34	57	0
			100	85	89	0
		Phosphormorpholidates				
7	CTAACCAGAGAGACC	1–15	4	43	43	14
			20	55	43	
			100	72	67	25
8	GCGTACTCACCAGTCGCCGC	280–299	4	65	52	
			20	92	98	
			100	100	100	
9	CGTACTCACCAGTCG	284–298	1	47	43	
			4	68	43	
			20	68	49	0
			100	64	63	0
10	ACACCCAATTCTGAAAATGG	5349–5368	4	59	57	
			20	91	86	
			100¶	(Toxic)		
11	CCCAATTCTGAAAAT	5351–5365	4	52	43	3
			20	47	46	
			100	49	75	0
		Phosphorbutylamidates				
12	GCGTACTCACCAGTCGCCGC	280–299	4	72	57	
			20	100	100	
			100	100	100	
13	CGTACTCACCAGTCG	284–299	1	53	23	
			4	59	23	
			20	47	34	
			100	58	43	14
		Phosphorpiperazidates				
14	GCGTACTCACCAGTCGCCGC	280–299	4	71	56	
			20	93	96	
			100¶	(Toxic)		
15	ACACCCAATTCTGAAAATGG	5349–5368	4	47	64	
			20	87	64	
			100¶	(Toxic)		

*Numbering of HIV RNA is taken from Muesing *et al.* (19); 280–299 contains a splice donor site and 5351–5365 contains a splice acceptor site.

†Assays are described in *Materials and Methods*. Values are means of duplicate determinations.

‡Percent reduction of the number of cells at 96 hr after a single addition of oligonucleotide (100 μg/ml) at time zero compared to control cells. No virus was used in this determination. Cell number was determined by staining with trypan blue and counting stained cells in a hemocytometer.

§Noncomplementary controls.

¶At 100 μg/ml, cell numbers fell below 50% of those in the infected control.

a crucial factor in determining activity among these nuclease-resistant derivatives, as they were equally active. Oligomer chain length had greater effect on antiviral activity, with the longer compounds being more potent. This behavior correlates with the melting temperatures of duplexes between these compounds and complementary unmodified DNA (unpublished results).

Our results on the inhibition of HIV-1 replication by phosphorothioates are in general agreement with those of Matsukura *et al.* (3). However, we did not observe any

Table 2. Anti-HIV activity of homooligodeoxynucleotides

Compound no.	Sequence	Conc., μg/ml	% inhibition		% reduction in cell no.
			Syncytia	p24	
		Phosphorothioates			
16	dA_{20}	4	0	36	
		20	26	36	
		100	88	91	
17	dC_{20}	4	58	36	
		20	98	91	
		100	100	95	
18	dG_{20}	4	51	91	
		20	100	100	
		100	100	91	
19	dT_{20}	4	60	82	
		20	100	93	
		100	100	100	
		Phosphormorpholidates			
20	dA_{15}	4	24	0	
		20	35	20	12
		100	83	70	21
21	dC_{15}	4	44	7	0
		20	35	20	0
		100	32	33	24
22	dG_{15}	4	31	0	0
		20	71	33	8
		100	93	80	67
23	dT_{15}	4	35	35	
		20	54	60	11
		100	98	97	76
		Phosphodiesters			
24	dA_{15}	4	9	40	
		20	33	75	4
		100	45	65	23
25	dC_{15}	4	54	0	0
		20	55	53	0
		100	56	80	5
26	dG_{15}	4	68	53	0
		20	75	67	0
		100	94	96	28
27	dT_{15}	4	42	55	16
		20	75	65	62
		100	91	80	78

Assays were performed as for Table 1.

correlation of inhibitory activity with G + C content. We have extended studies of the inhibitory effects of homopolymers to two other classes of homooligodeoxynucleotides, the morpholidates and parent phosphodiesters. Both proved to be as potent as similar antisense heterosequences but exhibited consistently greater toxicity in tissue cultures. Finding high potency was surprising, particularly in the case of the phosphodiesters where we have demonstrated previously that several noncomplementary control heterooligomers were inactive. Thus unmodified homooligomers appear to behave differently from the heterooligomers and may inhibit HIV by some mechanism other than antisense competitive hybridization.

It is known, for example, that homopolymers are better substrates than heteropolymers for reverse transcriptase (21). Hence, homooligomers might be competitive inhibitors of this enzyme or others involved in DNA synthesis. Also, degradation of a homooligonucleotide to mononucleotide would produce a much larger change in the intracellular pool of that nucleotide than would degradation of a comparable heterooligomer and also might occur at a different rate depending on the nature of the nucleases involved. In addition, homooligonucleotides might be able to associate rather nonspecifically with RNA by seeking out dispersed

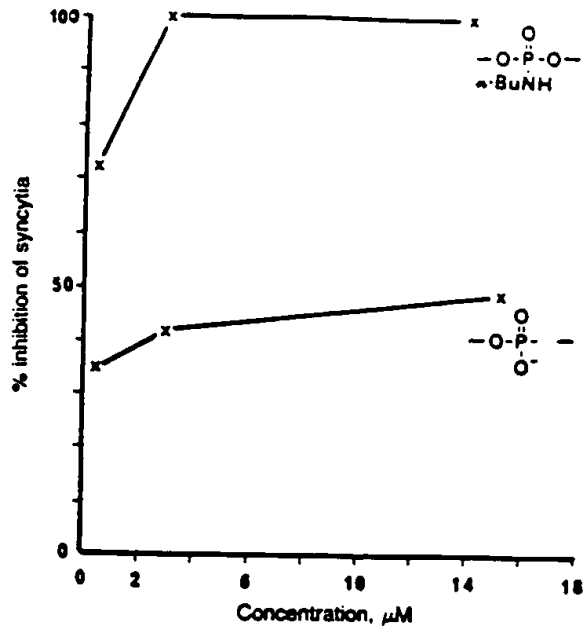

FIG. 2. Effect of internucleoside phosphate modification on the ability of an oligonucleotide to inhibit syncytium formation. The activity as a function of concentration of an oligodeoxynucleoside *N*-butylphosphoramidate (upper curve) is compared with an unmodified oligodeoxynucleotide of the same sequence (GCGTACTCA-CCAGTCGCCGC). This is complementary to sequence 280–299 in HIV RNA. All the phosphate modifications reported here as well as methylphosphonates (unpublished results) gave a similar enhancement in activity.

regions of limited but sufficient complementarity, possibly including some non-Watson–Crick base pairs. It has not been determined whether such interactions might, for example, lead to activation of ribonuclease H. These and other factors might account for the activity of homooligonucleotides and their tendency to inhibit cell division to a greater extent than the heterooligomers. While the antiviral properties of the homooligomers are worth pursuing, they are expected to be less selective in their action and hence potentially more toxic than true antisense oligomers.

In the case of the phosphorothioates, at low concentrations the noncomplementary control (compound 1 in Table 1) was inactive, whereas the antisense oligomers (compounds 3–6) were active. At higher concentrations, both the control and the antisense oligomers were equally active. Thus, at higher concentrations of this series of compounds, mechanisms of action other than competitive hybridization might be operational.

The one compound that was inactive at all concentrations was the self-complementary phosphorothioate (compound 2 in Table 1), which probably forms a double-stranded duplex (20). It has also been shown that methylation at N-3 of thymidine abolishes the activity of an oligonucleoside phosphorothioate (3). Thus, whatever mechanism of inhibition phosphorothioates may use in addition to or instead of antisense arrest, it might well require participation of the hydrogen-bonding centers on the bases. The availability of these would be restricted either by methylation or by base pairing. Alternatively, these hydrogen-bonding groups may be important for cellular uptake of the oligomers.

The phosphate-backbone modifications of oligomers described here produce an increase in HIV-1-inhibitory activity relative to the parent phosphodiesters. This effect was independent of the substitution or overall charge of the oligomers, which were all still capable of base pairing with complementary DNA. At higher concentrations, the phos-

phorothioates probably act by some other mechanism(s) in addition to antisense arrest. Homooligonucleotides were also active but may be a special case, as even unmodified diesters were active whereas noncomplementary heterosequences in this series are inactive. These studies point to the potential usefulness of the phosphate-backbone modifications in generating oligomers that may be more effective than unmodified oligomers either alone or in combination with other drugs in the therapy of patients with AIDS or AIDS-related complex. It is noteworthy that an oligonucleotide given 4 days after addition of the virus resulted in inhibition of HIV-1 expression after further incubation for 4 days, thus suggesting the potential effectiveness of these oligomers not only in preventing new infection but also in inhibiting HIV expression in previously infected individuals.

Addendum. Two noncomplementary sequences of oligonucleoside phosphormorpholidate were tested for their antiviral activity against HIV under the same conditions as described above. The two sequences were GCAGGCAAACCATTTGAATG and CGTAAGCAACAGTAGATCCT. Inhibition of HIV-induced syncytium formation was found at concentrations of 4, 20, and 100 μg/ml. The results were, respectively, 11%, 21%, and 30% for the first sequence and 15%, 15%, and 30% for the second sequence. Thus, inhibition from the noncomplementary sequences is less than that from the complementary ones described in the text. Preliminary acute-toxicity studies of oligonucleoside phosphormorpholidate in mice showed results similar to those for oligonucleoside phosphorothioate. No symptoms of toxicity were observed at dose levels of 2.5, 10, and 40 mg/kg of body weight, but with a dose of 150 mg/kg, two female animals died after 60 hr and two male animals were ill and recovered.

Toxicity tests were performed by University Laboratories, Inc., New Jersey. This work was supported by a grant from the G. Harold and Leila Y. Mathers Foundation, by Frederick Cancer Research Facility Contract FOD-0756-02, by National Cancer Institute Cancer Center Core Grant P30-12708-15, and by National Cooperative Drug Discovery Group for the treatment of AIDS Grant U 01 AI24846 from the National Cancer Institute and the National Institute of Allergy and Infectious Diseases.

1. Zamecnik, P. C. & Stephenson, M. L. (1978) *Proc. Natl. Acad. Sci. USA* 75, 280–284.
2. Zamecnik, P. C., Goodchild, J., Taguchi, Y. & Sarin, P. S. (1986) *Proc. Natl. Acad. Sci. USA* 83, 4143–4146.
3. Matsukura, M., Shinozuka, K., Zon, G., Mitsuya, H., Reitz, M., Cohen, J. S. & Broder, S. (1987) *Proc. Natl. Acad. Sci. USA* 84, 7706–7710.
4. Goodchild, J., Letsinger, R. L., Sarin, P. S., Zamecnik, M. & Zamecnik, P. C. (1988) in *Human Retroviruses, Cancer & AIDS: Approach to Prevention & Therapy*, UCLA Symposia on Molecular and Cellular Biology New Series, ed. Bolognesi, D. (Liss, New York), Vol. 77, pp. 423–438.
5. Miller, P. S., Agris, C. H., Aurelian, L., Blake, K. R., Murakami, A., Reddy, M. P., Spitz, S. A. & Ts'o, P. O. P. (1985) *Biochimie* 67, 769–776.
6. Lemaitre, M., Bayard, B. & Lebleu, B. (1987) *Proc. Natl. Acad. Sci. USA* 84, 648–652.
7. Agris, C. H., Blake, K. R., Miller, P. S., Reddy, M. P. & Ts'o, P. O. P. (1986) *Biochemistry* 25, 6268–6275.
8. Smith, C. C., Aurelian, L., Reddy, M. P., Miller, P. S. & Ts'o, P. O. P. (1986) *Proc. Natl. Acad. Sci. USA* 83, 2787–2791.
9. Stein, C. A. & Cohen, J. S. (1988) *Cancer Res.* 48, 2659–2668.
10. Zerial, A., Thuong, N. T. & Helene, C. (1987) *Nucleic Acids Res.* 15, 9909–9919.
11. Heikkila, R., Schwab, G., Wickstrom, E., Loke, S. L., Pluznik, D. H., Watt, R. & Neckers, L. M. (1987) *Nature (London)* 328, 445–449.
12. Holt, J. T., Redner, R. L. & Nienhuis, A. W. (1988) *Mol. Cell. Biol.* 8, 963–973.
13. Sproat, B. S. & Gait, M. J. (1984) in *Oligonucleotide Synthesis: A Practical Approach*, ed. Gait, M. J. (IRL, Oxford), pp. 83–115.
14. Froehler, B. C. & Matteucci, M. (1986) *Tetrahedron Lett.* 27, 469–472.
15. Garegg, P. J., Lindh, I., Regberg, T., Stawinski, J., Stromberg, R. & Henrichson, C. (1986) *Tetrahedron Lett.* 27, 4051–4054.
16. Froehler, B. C. (1986) *Tetrahedron Lett.* 27, 5575–5578.
17. Sarin, P. S., Taguchi, Y., Sun, D., Thornton, A., Gallo, R. C. & Oberg, B. (1985) *Biochem. Pharmacol.* 34, 4075–4078.
18. Sarin, P. S., Sun, D., Thornton, A. & Muller, W. E. G. (1987) *J. Natl. Cancer Inst.* 78, 663–666.
19. Muesing, M. A., Smith, D. H., Cabradilla, C. D., Benton, C. V., Lasky, L. A. & Capon, D. J. (1985) *Nature (London)* 313, 450–458.
20. Stein, C. A., Subasinghe, C., Shinozuka, K. & Cohen, J. S. (1988) *Nucleic Acids Res.* 16, 3209–3221.
21. Allaudeen, H. S. (1980) in *Inhibition of RNA & DNA Polymerases*, ed. Sarin, P. S. & Gallo, R. C. (Pergamon, New York), pp. 1–26.
22. Wickstrom, E. L., Bacon, T. A., Gonzalez, A., Freeman, D. L., Lyman, G. H. & Wickstrom, E. (1988) *Proc. Natl. Acad. Sci. USA* 85, 1028–1032.
23. Goodchild, J., Agrawal, S., Civeira, M., Sarin, P. S., Sun, D. & Zamecnik, P. C. (1988) *Proc. Natl. Acad. Sci. USA* 85, 5507–5511.